

Asthma is associated with single-nucleotide polymorphisms in *ADAM33*

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Summary

Background The *ADAM33* gene has recently been associated with asthma and bronchial hyper-reactivity. It codes for a disintegrin and metalloproteinase that triggers intra- and extracellular signalling by protein shedding.

Objective We examined whether polymorphisms in *ADAM33* are associated with asthma and related traits in two German populations.

Methods We genotyped 15 intragenic single-nucleotide polymorphisms (SNPs) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of allele-specific primer extension products. The transmission disequilibrium test was used for association analysis in the German asthma family study. Additionally, we tested for association of these SNPs in a case-control sample from the European Community Respiratory Health Study using Armitage's trend test.

Results In both studies, we found SNPs that were significantly associated with asthma and related traits. In the family study, significant associations were observed for the SNPs F+1, ST+4 and ST+5 (with the lowest *P*-value for F+1, *P* = 0.005). Remarkably, this association is seen even in the absence of linkage with two microsatellite markers from a previous genome scan either 3.1 million bases (Mb) up- or 5.6 Mb downstream. In the case-control study, SNP ST+7 (*P* = 0.008) was significantly associated with asthma. Some of these SNPs overlapped with those found to be associated with elevated total IgE levels and bronchial hyper-responsiveness.

Conclusion This study replicates the recently published association between asthma and *ADAM33* gene variants. However, most of the associated SNPs were at non-identical positions in the German, UK and US samples. As linkage disequilibrium is high among the tested SNPs, and there is no known functional polymorphism, either not-tested variants in *ADAM33*, unknown regulatory elements or a gene in close proximity is responsible for this association.

Keywords *ADAM33*, association study, asthma, bronchial hyper-responsiveness, case-control, family study

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Introduction

Asthma is a chronic inflammatory disorder with increasing prevalence over the past decades. It is characterized by variable airflow obstruction, inflammation of the airways and bronchial hyper-responsiveness (BHR). Whereas many environmental factors are known to trigger asthma, several segregation studies also showed evidence for a genetic predisposition to develop asthma. The clarification of the genetic basis of this disease has been complicated due to the clinical and genetic heterogeneity of asthma-associated

phenotypes. Although atopy is one of the strongest risk factors associated with asthma, neither atopic predisposition nor eosinophilia may account for the organ-specific expression of the disease. This concept has been supported by genetic studies that have demonstrated different patterns of inheritance for atopy and BHR [1]. In a previous genome scan, asthma and BHR have been linked to a ~ 4 million base (Mb) interval on chromosome 20p13 [2]. This genomic region harbours a member of the membrane-anchored *ADAM* (a disintegrin-like and metalloproteinase-containing) protein family. By using a panel of 135 single-nucleotide polymorphisms (SNPs) in a case-control study with 130 asthmatics and 217 controls, van Eerdekewegh et al. [2] identified seven SNPs in the *ADAM33* gene that were significantly associated with asthma. The recently discovered *ADAM33* exhibits a complex preserved domain structure containing extracellular catalytic domains, the transmembrane domain and the cytoplasmic tail [2, 3]. According to phylogenetic analysis, the protein *ADAM33* seems to possess

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proteolytic activity [3]. Due to exon skipping, *ADAM33* has at least two isoforms that are expressed in different cell types from lung tissues [2, 3].

In addition to several inflammatory features, asthma is accompanied by an airway remodelling process. The changes in the airway structure and the following airway narrowing results from a variety of interdependent inflammatory and remodelling processes [4]. Metalloproteases such as *ADAM33* might contribute to airway remodelling by the release of several inflammatory mediators and growth factors as well as the degradation of extracellular matrix components. Hence, *ADAM33* seems to be a reasonable candidate gene for asthma.

We examined whether polymorphisms in *ADAM33* are associated with asthma. We analysed 15 SNPs in this gene that were determined as significant in the UK and US samples [2]. In this study, we used the transmission disequilibrium test for association analysis in 171 German families with at least two affected siblings. In addition, we also performed an association analysis in an independent case-control sample from two German European Community Respiratory Health Survey (ECRHS) centres.

Methods

Study population and phenotyping

The German asthma sib pair families were collected in 26 paediatric centres in Germany and Sweden for an initial genome-wide linkage scan [5]. In these families, at least two children were required with confirmed clinical asthma, while prematurity or low birth weight of the children were excluded, along with any other severe pulmonary disease. All affected children over the age of 3 years had a history of at least 3 years of recurrent wheezing and with no other airway disease diagnosed. On the first home visit, a complete pedigree of the family was drawn and information was collected in a questionnaire. Participants were examined for several associated phenotypes. Pulmonary function tests were performed by forced flow volume tests. Bronchial challenge was carried out with increasing doses of methacholine [5]. The dose necessary for a 20% fall of forced expiratory volume in 1 s is given as PD_{20} and probands with a $PD_{20} \leq 2$ mg were considered as bronchial hyper-reactive in this analysis (BHR). Total IgE was determined with an ELISA (Pharmacia Diagnostics, Uppsala, Sweden). IgE levels were considered as elevated at values greater than 100 kU/mL. The original families [5] have now been expanded to nearly 250 families, of which 218 families remained under analysis after exclusion of those with incomplete clinical data, missing DNA samples, having identical twins or showing more than two non-segregating markers. In all, 732 individuals from 171 families could be genotyped in this study.

Each study participant signed a consent form. All study methods were approved in 1995 by the ethics commission of 'Nordrhein-Westfalen' and again in 2001 by 'Bayerische Landesärztekammer München'.

As a second sample, we used a nested case-control study within the follow-up of an ongoing cohort study. The first stage of the ECRHS was performed from 1990 to 1992 in 22

countries [6]. This analysis includes only individuals from the German centres Hamburg and Erfurt. Random samples of 731 subjects in the age range of 20–48 years were selected in Erfurt and 900 in Hamburg in stage I data collection. From this sample, 590 subjects were re-examined in stage II starting in 2000 for a detailed questionnaire and an extended examination comprising spirometric measurements, methacholine or bronchodilator inhalation tests, and determination of total and specific IgE. This study protocol was approved by several local ethics committees.

Pulmonary function testing comprised baseline spirometry and the determination of flow-volume curves (Masterlab, Jaeger, Wuerzburg, Germany). All measurements as well as methacholine challenges were performed strictly adhering to the ECRHS protocol and quality standards [6]. The methods and exclusion criteria have been described in detail elsewhere [7, 8]. The same criterion was used for BHR as described above. Total IgE was measured using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Genotyping could be performed in 547 individuals, of whom 48 were asthmatics.

DNA isolation and genotyping

DNA was isolated from peripheral white blood cells using Quiamp (Quiagen, Germany) or Puregene isolation kits (Gentra Systems, Minneapolis, MN, USA). Of the 37 polymorphisms in the *ADAM33* gene typed in the UK/US case-control sample by van Eerdewegh et al., we selected 15 that have been shown to be associated with asthma or BHR in this sample. Of these SNPs, six were exonic and nine intronic (Table 1). Genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of allele-specific primer extension products generated from previously amplified DNA sequences (MassARRAYTM, SEQUENOM Inc., San Diego, CA, USA). Primers were obtained from Metabion GmbH (Planegg-Martinsried, Germany). Detailed assay description and primer information are available at <http://cooke.gsf.de/wjst/paper/2003ClinExpAllSuppl/>

Data handling and statistical analysis

Clinical data and genotypes were transferred to an SQL2000 database. All analyses were performed using SAS for windows V8 (SAS Institute, Cary, NC, USA). For each SNP, the distribution of genotypes in pseudo-controls (created by combining the parental alleles not transmitted to the first child with asthma; family study) and controls (individuals without asthma; case-control study) were tested for deviations from Hardy-Weinberg equilibrium by a χ^2 -test. We tested for association between *ADAM33* gene variants and the phenotypes asthma, BHR, elevated total IgE, and asthma plus BHR. An extension [9] of the method of Zhao et al. [10] was used for association analysis at a single-marker locus or of multi-locus haplotypes with any phenotypes in the families. To compare single-marker genotype distributions between cases and controls within the follow-up of the ECRHS, Armitage's trend test [11] was applied. Multi-locus haplotype frequencies in cases and controls were compared by a likelihood ratio test. Haplotypes were analysed for two or

Table 1. Association of single-nucleotide polymorphisms (SNPs) in the *ADAM33* gene with asthma; for comparison, the recently published results [2] were included

SNP*	Location	Allele†	UK case-control [2] Pca/Pco‡ P-value	US case-control [2] Pca/Pco‡ P-value	US/UK families [2] T/NT§ P-value	German case-control Pca/Pco‡ P-value	German families T/NT§ P-value
F+1	Intron	<u>G</u> /A	74.2/64.1 0.0295	57.4/67.4 0.2401	NA	69.8/60.6 0.0829	79/45 0.0048
I1	Catalytic domain	<u>G</u> /A	89.2/83.7 0.1094	70.4/87.2 0.0105	NA	79.1/81.2 0.6658	34/21 0.1425
M+1	Intron	<u>G</u> /T	91.8/87.0 0.1059	77.8/92.0 0.0116	NA	85.6/86.1 0.9033	54/53 1.0000
Q-1	Intron	<u>C</u> /T	92.3/86.1 0.0419	87.0/83.1 0.6654	NA	89.1/85.2 0.4612	41/29 0.1825
S1	Transmembrane domain	<u>G</u> /A	95.2/89.4 0.026	92.3/89.6 0.7873	37/20 0.033	91.3/90.6 0.8384	42/32 0.2325
S2	Transmembrane domain	<u>G</u> /C	84.0/72.9 0.0041	64.8/75.3 0.1571	NA	71.3/70.6 0.9048	87/62 0.0645
S+1	Intron	<u>A</u> /T	50.5/53.1 0.6346	60.9/48.1 0.1345	NA	48.6/54.8 0.3184	87/77 0.4825
ST+4	Intron	<u>A</u> /C	59.2/48.1 0.0191	63.5/57.1 0.515	NA	67.4/59.6 0.1624	80/108 0.0450
ST+5	Intron	<u>T</u> /C	50.0/44.4 0.2273	44.4/50.0 0.5287	NA	50.0/42.6 0.2044	107/148 0.0123
ST+7	Intron	<u>G</u> /A	86.4/79.5 0.0535	83.3/75.7 0.3413	NA	89.6/78.1 0.0078	72/52 0.1050
T1	Cytoplasmatic domain	<u>T</u> /C	86.8/91.3 0.1473	75.9/92.2 0.003	43/27 0.072	85.4/84.5 0.8537	43/46 0.8750
T2	Cytoplasmatic domain	<u>T</u> /C	8.2/10.5 0.4279	20.4/7.4 0.0188	NA	15.5/15.2 0.9429	47/45 0.9100
T+1	Intron	<u>C</u> /T	91.1/86.9 0.2211	80.0/92.0 0.0334	NA	85.4/85.2 0.9470	45/51 0.6250
V-1	Intron	<u>C</u> /A	93.8/86.4 0.0105	87.0/82.9 0.5262	43/27 0.072	92.2/86.1 0.1474	14/11 0.6875
V4	3'-UTR	<u>C</u> /G	83.7/75.4 0.0328	83.3/79.2 0.5583	73/55 0.13	81.3/77.8 0.4834	50/34 0.0920

NA, not available.

*SNP identifier as in [2]. †Reference allele underlined. ‡Prevalence in cases/prevalence in controls. §Transmitted/untransmitted allele.

Bold indicates $P < 0.05$.

three adjacent SNPs. The standardized linkage disequilibrium (LD) coefficient D' [12] was calculated for each pair of SNPs on the basis of estimates for the frequencies of two-locus haplotypes in parents (family study) and the whole sample (case-control study). As there was *a priori* hypothesis with all tested SNPs, we did not adjust significance levels for multiple testing.

Results

We analysed 15 SNPs in the *ADAM33* gene in a German family-based study and an independent population-based case-control study. Genotypes could be determined for 390 children, of whom 89% had physician-diagnosed asthma. Information regarding BHR was available for only 53% of all children; however, only 11 children with known BHR were not affected by asthma. Thus, the samples characterized by the phenotypes 'BHR' or 'asthma and BHR' were nearly identical. Because van Eerdewegh et al. [2] obtained the strongest association for 'asthma plus BHR' we also tested

for this phenotype. Furthermore, 276 children showed elevated total IgE.

The case-control sample comprised 547 individuals, of whom 9% were asthmatics, 91 individuals were bronchial hyper-reactive, and 128 individuals showed elevated IgE levels. The phenotype 'asthma and BHR' was not analysed here, because only 14 subjects were affected by asthma and were hyper-reactive at the time of examination.

In the single-locus analysis (Table 1), we observed three significant P -values for asthma in the family study at F+1 ($P = 0.005$), ST+4 ($P = 0.045$) and ST+5 ($P = 0.012$). Some of these SNPs overlapped with those found to be associated with elevated total IgE (F+1, $P = 0.034$; ST+5, $P = 0.032$), BHR (F+1, $P = 0.0096$; S2, $P = 0.010$) or 'asthma plus BHR' (F+1, $P = 0.0096$; S2, $P = 0.008$). To confirm these findings, we also tested the same SNPs in the ECRHS study sample. In this study, only ST+7 reached significance concerning asthma (Armitage's trend $P = 0.008$). BHR was associated with ST+5 ($P = 0.003$) and IgE with ST+7 ($P = 0.006$).

Two- and three-locus haplotype analysis revealed several significant associations in the family study (Table 2), with the lowest P -values observed for BHR (S2/S+1 and S1/S2/S+1;

Table 2. Haplotype analysis of *ADAM33* (SNP haplotypes (two- and three-locus) significant at $P < 0.05$ in German families; indicated in bold)

SNP #1*	SNP #2*	SNP #3*	Asthma <i>P</i> -value	BHR <i>P</i> -value
S2	S+1		0.343	0.004
S+1	ST+4		0.565	0.008
ST+4	ST+5		0.006	0.405
Q-1	S1	S2	0.105	0.004
S1	S2	S+1	0.438	0.004
S2	S+1	ST+4	0.980	0.004

SNP, single-nucleotide polymorphism; BHR, bronchial hyper-responsiveness.
*SNP identifier as in [2].

$P = 0.004$). Comparing two-locus haplotype frequencies between cases and controls, the most significant associations were found for asthma (ST+4/ST+7; $P = 0.00013$).

The overall LD measured as D' was high between most of the tested polymorphisms in the *ADAM33* gene. As displayed in Fig. 1, LD was very similar in both samples.

Discussion

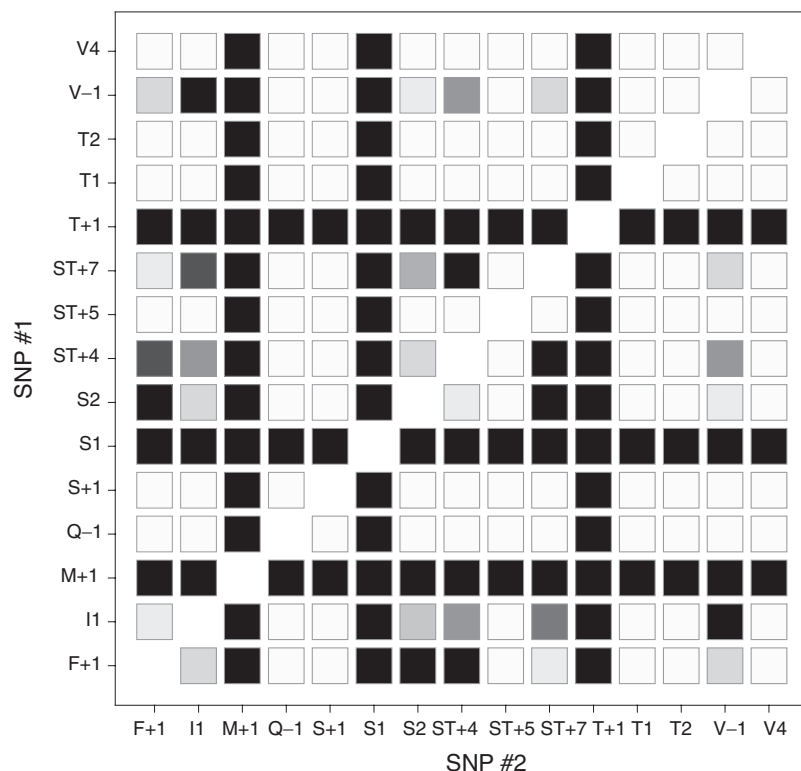
Currently, asthma is thought to result from the combined effects of allergic airway inflammation and dysfunction of the airways. While most studies have focused on candidate genes controlling the allergic inflammation, we report here the association to the *ADAM33* gene, which may influence airway function rather than affect the immune response. The 15 tested SNPs were chosen because of the recently found association in a UK/US case-control sample [2]. In two

independent studies, a case-control study as well as a family study, we observed significant association of several SNPs with asthma and related phenotypes.

This supports the results by van Eerdewegh et al.[2], who found the strongest linkage at 20p13 near the genomic marker D20S482 for the phenotype asthma and BHR. We have observed only weak linkage at 20p12 in our previous linkage scan [5], while the updated genome scan of the families (unpublished data) did not reveal any linkage in the two most closely situated to *ADAM33* (D20S117, distance ~ 3.1 Mb, lod 0.11; marker D20S917, distance ~ 5.6 Mb, lod 0.22). This is therefore a remarkable example of association in the absence of linkage.

The association with asthma was also reproduced in an independent population-based study; however, most of the associations were at different positions (Table 1). The different LD pattern in the US/UK sample and the German population may account for the observed heterogeneity between the previous study and our own results. Differences were even seen in the primary study between the US and UK samples. While our case-control sample is derived from a population-based study, the asthma families were primarily selected on the basis of asthma severity. This sampling bias may explain that significant SNPs were not identical despite the same population background.

Apart from the inflammatory process, structural changes of the airways due to airway remodelling play an important role in the development of airway obstruction and BHR associated with asthma. Contrary to the general concept that remodelling in asthma is secondary to long-standing inflammation, this process starts early in life, with pathological features being present before onset of the disease [13, 14]. The interaction of different cellular and molecular mechanisms

**Fig. 1.** Linkage disequilibrium in the *ADAM33* gene estimated from the German parents including one child (upper left) and by the German case-control study (lower right). Black indicates low (0) and white high (1) D' -values.

results in abnormal mesenchymal cell proliferation, changes in the extracellular matrix and changes in the composition of the airway wall [14]. While matrix metalloproteinases (MMPs) have attracted much attention in the past due to their role in normal physiology as well as in a variety of diseases such as cancer, rheumatoid arthritis and asthma [15, 16], the interest in the *ADAM* subfamily heightened when their proteolytic function in the shedding of numerous membrane-anchored molecules was discovered. For example, the release of TNF- α is mediated by *ADAM17* [17]. Since *ADAM33* was characterized only earlier this year [3, 18], its biological function, and thus its role in asthma pathogenesis, remains unclear. As reported by van Eerdewegh et al. and others [2, 19], *ADAM33* is expressed in lung fibroblasts and bronchial smooth muscle cells, but not in the bronchial epithelium [2]. Due to its expression profile, *ADAM33* is suspected of being associated with airway remodelling observed in asthmatics. Given the function of *ADAMs* in membrane shedding, this proteinase may contribute to the proliferation of smooth muscle cells by the release of cytokines or growth factors. Polymorphisms in *ADAM33* may lead to impaired or enhanced shedding of these molecules, thus affecting proliferation and/or inflammation in the airways. The development of BHR may be the consequence of this inflammatory and remodelling process.

In contrast to the proposed biological function of *ADAM33* in asthma pathogenesis, none of the significant variants seem to have an obvious functional relevance for the *ADAM33* protein [3]. Although some of the known 98 SNPs in *ADAM33* may affect transcription factor binding sites, disrupt potential exon-splice enhancer or influence RNA stability (unpublished own annotation data), there are no functional data available so far that support any SNPs to have a disease-causing effect.

Since LD in this region is expected to be high (see Fig. 1) and extends up to ~ 185 kb on both sides of *ADAM33* [2], it is also possible that other candidate genes or regulatory elements on the adjacent LD block like *ATRN*, *GFR44*, *SN* or *C200RF* are responsible for the observed association. In addition to *ADAM33*, single SNPs in *GFR44* and *SN* also reached significance in the UK/US sample.

In conclusion, this study is the first report that could replicate findings on the recently published association between asthma and *ADAM33* gene variants in two independent samples. To understand the consequences of genetic variation, further investigation of the biological function of *ADAM33* is necessary. Since *ADAM33* is the first described gene possibly affecting the disease organ in particular, this might provide new diagnostic or therapeutic strategies.

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References

- 1 Jansen DF, Rijcken B, Schouten JP et al. The relationship of skin test positivity, high serum total IgE levels, and peripheral blood eosinophilia to symptomatic and asymptomatic airway hyperresponsiveness. *Am J Respir Crit Care Med* 1999; 159:924–31.
- 2 van Eerdewegh P, Little RD, Dupuis J et al. Association of the *ADAM33* gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418:426–30.
- 3 Yoshinaka T, Nishii K, Yamada K et al. Identification and characterization of novel mouse and human *ADAM33s* with potential metalloprotease activity. *Gene* 2002; 282:227–36.
- 4 Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000; 161:1720–45.
- 5 Wjst M, Fischer G, Immervoll T et al. A genome-wide search for linkage to asthma. German Asthma Genetics Group. *Genomics* 1999; 58:1–8.
- 6 Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994; 7:954–60.
- 7 Nowak D, Heinrich J, Jorres R et al. Prevalence of respiratory symptoms, bronchial hyperresponsiveness and atopy among adults: west and east Germany. *Eur Respir J* 1996; 9:2541–52.
- 8 Wassmer G, Jorres RA, Heinrich J, Wjst M, Reitmeir P, Wichmann HE. The association between baseline lung function and bronchial responsiveness to methacholine. *Eur J Med Res* 1997; 2:47–54.
- 9 Knapp M. A family-based test for association in the presence of linkage with multiple tightly linked markers in nuclear families with multiple affected children. *Genet Epidemiol* 2001; 21:162.
- 10 Zhao H, Zhang S, Merikangas KR et al. Transmission/disequilibrium tests using multiple tightly linked markers. *Am J Hum Genet* 2000; 67:936–46.
- 11 Sasieni PD. From genotypes to genes: doubling the sample size. *Biometrics* 1997; 53:1253–61.
- 12 Lewontin RC. On measures of gametic disequilibrium. *Genetics* 1988; 120:849–52.
- 13 Warner JO, Pohunek P, Marguet C, Clough JB, Roche WR. Progression from allergic sensitization to asthma. *Pediatr Allergy Immunol* 2000; 11 (Suppl. 13):12–4.

- 14 Cokugras H, Akcakaya N, Seckin S, Camcioglu Y, Sarimurat N, Aksoy F. Ultrastructural examination of bronchial biopsy specimens from children with moderate asthma. *Thorax* 2001; 56:25–9.
- 15 Chiappara G, Gagliardo R, Siena A et al. Airway remodelling in the pathogenesis of asthma. *Curr Opin Allergy Clin Immunol* 2001; 1:85–93.
- 16 Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol* 2002; 3:207–14.
- 17 Ohbayashi H. Matrix metalloproteinases in lung diseases. *Curr Protein Pept Sci* 2002; 3:409–21.
- 18 Black RA, Rauch CT, Kozlosky CJ et al. A metalloproteinase disintegrin that releases tumour-necrosis factor- α from cells. *Nature* 1997; 385:729–33.
- 19 Gunn TM, Azarani A, Kim PH, Hyman RW, Davis RW, Barsh GS. Identification and preliminary characterization of mouse *ADAM33*. *BMC Genet* 2002; 3:2.